**BIO 510 Quiz 1 WITH ANSWERS**

1. (2 pts) DNA ligase differs from EcoRI in that its enzymatic reaction requires (circle all correct responses)
   1. Magnesium (in both)
   2. ATP (ligase only)
   3. Lead acetate (in neither)
   4. DNA substrate (required by both)
   5. Ethanol (in neither)
2. (2 pts) If the nucleotide composition of a DNA molecule is random (that is, no bias toward or against any of the 4 bases), on average how frequently would each of the restriction enzymes cut (for instance, 1/256, that is, once every 256 base pairs)? Only the top strand of the restriction site is given, in parenthesis is the enzymes name.
   1. 5’ GAATTC 3’ (EcoRI) 46 = 1/4096
   2. 5’ GATC 3’ (Sau3A) 44 = 1/256
   3. 5’GTYRAC 3’ (HincII) 44 X 22 = 1/1024
   4. GACGCNNNNN (HgaI) 45 X 15 1/1024

Note: Y = C or T; R=A or G; N=A,C,G, or T

1. (6 pts total) After you digested the pTZ18u(or19u) vector with EcoRI and HindIII you added a mixture of phenol/chloroform/isoamyl alcohol (PCI) mixture to your digestion.
   1. (2 pts) Tell why you did this (what was the value to your experiment in doing this step)? The goal was to remove the restriction enzyme from the DNA mixture. This was important as the next step was to ligate this DNA with the insert. If the EcoRI or HindIII was still present it would compete with the ligase activity (i.e., ligase would join the ends and then the EcoRI would re-cleave the DNA).
   2. (2 pts) What biochemical principles underlie the basis for this technique (that is, why does it work)? This is a two phase partition between an aqueous layer and an organic layer. The hydrophilic nature of DNA makes it partition in the hydrophilic layer while the protein is found in the organic layer or at the interface between the organic and aqueous layers.
   3. (2 pts) What role did each of the organic compounds contribute to the PCI function?
      1. Phenol – denatures and solubilizes proteins
      2. Chloroform – denatures protein
      3. Isoamyl alcohol – helps create a distinct interface between the aqueous and organic layers